St Mary's University Twickenham London
Institute of Education

St Mary's secondary science PGCE biology practical booklet microbial action

2025-2026

Name _____

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Contents page

General information

Over these practical days you will be completing a series of biology required practicals.

The intended outcomes of these sessions are:

- To ensure you are familiar with example required practicals

- To identify sources for practical procedure
- To reflect on how you may teach/introduce required practicals within a wider unit.
- To evaluate resources available.

In each session there will be an opportunity to record results and answer set questions. I would strongly advise you complete each of the tasks set to get a feel of the expectations upon students and any key skills you may need to reinforce.

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Working scientifically skill setting up simple enquiries and making observations

Pre-lab activity to be completed on the practical day.

In this practical you are going to test the action of a variety of anti-bacterial agents.
Use the details below to create a detailed method for conducting your experiment. Any record sheet will include headings as appropriate to your specific teaching/ learning objectives for this work, and relevant to your examining specification

Investigating microbial action

Students could make their own pour plates (see Standard technique Making a pour plate), or you could demonstrate how to make one and provide plates freshly prepared by technical staff.

There is scope for developing or assessing a range of experimental and investigative skills (including students' ability to make a risk assessment) if this is set up as an open-ended student-led investigation. Students could develop their own hypotheses to test, based on ideas introduced by the teacher and researched further on-line.

Students could compare:

- different brands of similar products
- the effects of extracts from different plant materials
- the effect of changing concentration on the effectiveness of anti-microbials
- the effect of anti-microbials on different microbes.

Apparatus and Chemicals

For the class – set up by technician/ teacher:

Microbial broth culture – such as *Bacillus subtilis*, *Escherichia coli* or *Micrococcus luteus*

Sterile Petri dishes, 1 or 2 per group

Sterile forceps, 1 or 2 per group

Disinfectant solution, 1 discard beaker per group

For each group of students:

Bunsen burner

Nutrient agar – in a sterile McCartney/ Universal bottle, 1

Materials with anti-microbial action, according to the students' hypotheses

Paper discs, Whatman antibiotic assay paper discs, or new filter/ chromatography paper cut with a hole punch then sterilised by autoclaving, 4-8

Sterile forceps

Adhesive tape

Marker pen

Health & Safety and Technical notes

Carry out a full risk assessment before planning any work in microbiology (see **Note 1** for more details).

Check the standard techniques for more details of Making up nutrient agars, Pouring an agar plate, Making a spread or 'lawn' plate and Incubating and viewing plates.

Some strains of the bacteria listed above have been associated with health hazards. Use microbes from the 'safe micro-organisms' list provided by SGM (Appendix 2, page 31 in Basic Practical Microbiology) or CLEAPSS (Table 15.2 in Section 15 of the Laboratory Handbook). These microbes present the minimum risk given good practice. Recognised educational suppliers should provide safe strains. The *E. coli* K12 strain is not thought to have any harmful effects and is preferable because of its more rapid and consistent growth.

Ensure that no members of the group are debilitated or taking immuno-specific medication, as this may increase their risk of infection by the bacteria used.

When using any commercial products, refer to manufacturers' guidelines, avoid contact with eyes, and limit skin contact.

Take particular care that ethanol used to sterilise instruments or make plant extracts is kept away from lit Bunsen burners. See CLEAPSS Hazcard: ethanol is HIGHLY FLAMMABLE, and IDA is HARMFUL.

Read our standard health & safety guidance

1 Before embarking on any practical microbiological investigation carry out a full risk assessment. For detailed safety information on the use of micro-organisms in schools and colleges, refer to *Basic Practical Microbiology – A Manual* (BPM) which is available, free, from the Society for General Microbiology (email education@sgm.ac.uk) or go to the safety area of the SGM website (http://www.microbiologyonline.org.uk/teachers/safety-information) or refer to the CLEAPSS Laboratory Handbook.

2 Making plant extracts: Crush 3 g of plant material with 10 cm^3 of ethanol (IDA). The CLEAPSS Hazcard describes this as HIGHLY FLAMMABLE (flash point 13 ºC) and HARMFUL (because of presence of methanol). Shake from time to time for 10 minutes.

3 Preparing Whatman antibiotic assay paper discs: Soak the paper discs in the extract or solution to be tested. Remove them with sterile forceps. Allow them to dry on an open, sterile Petri dish, next to a lit Bunsen burner to create an up draught to limit aerial contamination. If the extract contains ethanol, make sure it has evaporated completely before using. Consider the value of using a control disc soaked in ethanol (and dried as above).

Procedure

SAFETY:
Students will need to observe basic hygiene rules and follow aseptic techniques. They need to be aware of what to do with spills and how to dispose of cultures and contaminated equipment.

Your risk assessment should take into account your students' behavior and all plates should be stopped by treatment with methanal if students cannot be trusted to leave the plates closed, and not to remove them from the laboratory.

Preparation

a Discuss antimicrobial action of different substances with the students.

b Make a pour plate using the following method:

In a pour plate, a small amount of inoculum from a broth culture is added by pipette to the centre of a Petri dish. Cooled, but still molten, agar medium in a test tube or bottle is then

poured into the Petri dish. The dish is then rotated gently, or moved back and forth (first N-S, then NW-SE, then NE-SW), to ensure that the culture and medium are thoroughly mixed, and the medium covers the plate evenly.

Pour plates allow micro-organisms to grow both on the surface and within the medium. Most of the colonies grow within the medium and are small and may be confluent. The few colonies that grow on the surface are of the same size and appearance as those on a streak plate.

These plates could then be used to test the anti-microbial effects of various substances. For more information see Investigating antimicrobial action.

Otherwise, plates such as this could be part of the investigation of the population of bacteria in a sample. Culturing serial dilutions in this way permits a calculation of the population size of a bacterial sample. If the dilution and volume of inoculum, usually 1 cm^3, are known, the **viable count** of the sample per cm^3 can be determined. The viable count is the number of bacteria or clumps of bacteria per cm^3. The dilutions chosen must produce between 30 and 100 separate countable colonies. (See also Standard technique: Making serial dilutions)

Health & Safety and Technical notes

Read our standard health & safety guidance

1 Use a water bath at 50 °C to store bottles of molten agar.
2 Take care not to contaminate the molten agar in the bottles with water from the water bath. To avoid contamination, ensure:
i that the water in the water bath is at the right depth
ii that the bottles are kept upright
iii that the outsides of the bottles are wiped before they are used.
3 In an evenly spread pour plate, the base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.
4 CLEAPSS Laboratory handbook section 15.2.12 suggests some alternative methods of making a pour plate, and considers their pros and cons.

Procedure

Inoculation using a Pasteur pipette
At all times, hold the pipette as still as possible.
i) Loosen the cap/ cotton wool plug of the bottle containing the inoculum.
ii) Remove the sterile Pasteur pipette from its container, attach the teat and hold in your right hand.
iii) Lift the bottle/ test tube containing the inoculum with your left hand.
iv) Remove the cap/ cotton wool plug with the little finger of your right hand.
v) Flame the bottle/ test tube neck.

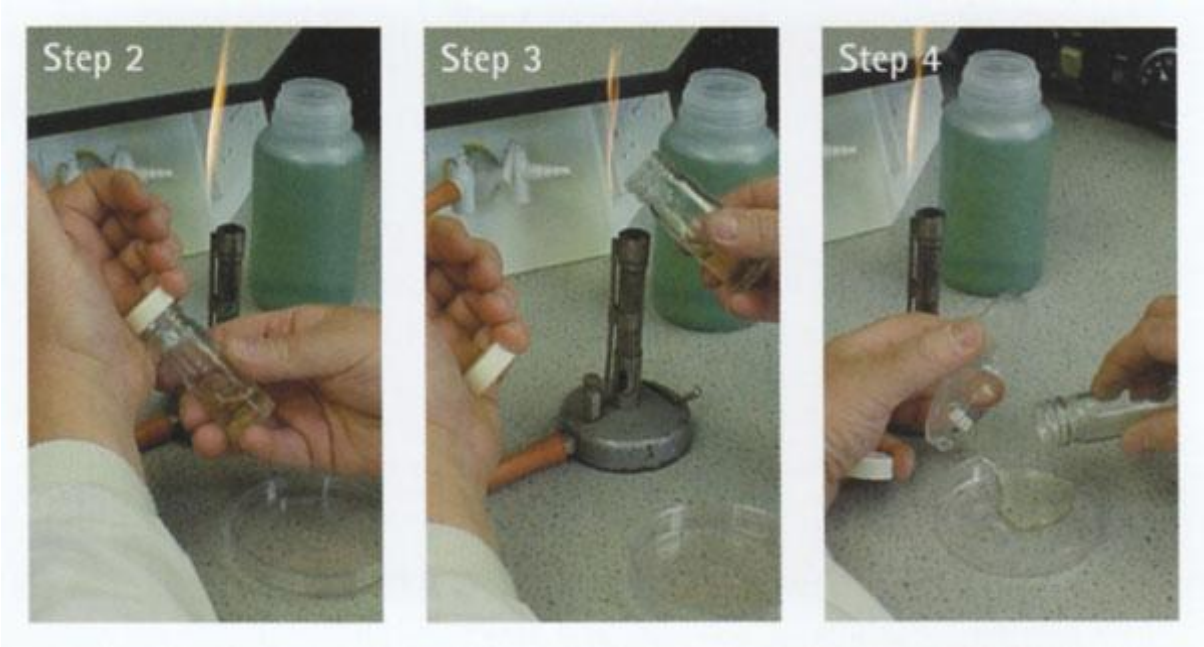
vi) Squeeze the teat bulb of the pipette very slightly. Put the pipette into the bottle/ test tube and draw up the required volume of the culture. Do not squeeze the teat bulb of the pipette after it is in the broth as this could cause bubbles and possibly aerosols.
vii) Remove the pipette and flame the neck of the bottle/ test tube again. Replace the cap/ cotton wool plug.
viii) Place the bottle/ test tube on the bench or in its rack.

Inoculating the Petri dish

i) Lift the lid of the Petri dish slightly with your right hand and insert the pipette into the Petri dish. Gently release the required volume of inoculum onto the centre of the dish. Replace the lid.
II) Put the pipette into a discard pot.

Pouring the plate

i) Collect a bottle of sterile molten agar from the water bath (note 1 and 2).
ii) Hold the bottle in your right hand. Remove the cap with the little finger of your left hand. Flame the neck of the bottle.
iii) Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish. Replace the lid.
iv) Flame the neck of the bottle and replace the cap.



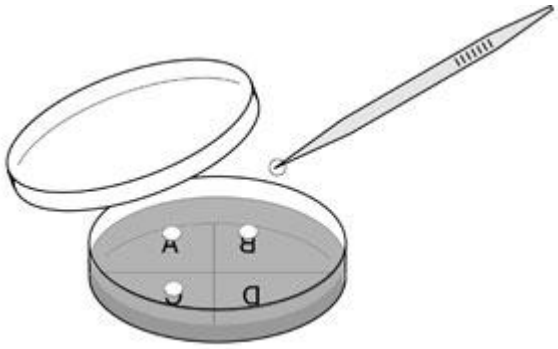
v) Move the dish gently to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly (note 3). Either move the dish in three directions: first N-S, then NW-SE, then NE-SW, or rotate it until the medium and inoculum are well-mixed and cover the base of the dish.

vi) Allow the plate to solidify.

vii) Tape the plate closed and incubate in an inverted position

viii) Explain or demonstrate how to make a plant extract. (**Note 2**)

ix) Explain or demonstrate how to use Whatman antibiotic assay paper discs to assess microbial action. (**Note 3**)



x) Allow students time to prepare a hypothesis to test, devise a detailed method and write a risk assessment.

Investigation

i) Each group will need to prepare a pour plate seeded with bacteria, or have one provided.

ii) Make up the solutions to test. You can make a plant extract as described in note 2. It might be interesting to test each substance at different dilutions.
Graduated pipettes are numbered as a burette – with liquid reaching the zero mark when the pipette is full. This allows you to record exactly how much liquid you have dispensed from the pipette. A procedure for serial dilutions is outlined below.

Procedure

SAFETY: Check any relevant hazcards or safety literature for your solvent and the substance under test.

a Label five appropriate tubes (test tubes or boiling tubes – according to how much solution you are making): 1, x10, x100, x1000, x10000
b Measure 11 cm^3 of your starting solution into the first tube (labelled 1).
c Use a 10 cm^3 syringe or pipette to put 9 cm^3 of solvent (such as distilled water) into each of the other tubes.
d Mix the contents of the first test tube thoroughly.
e Remove 1 cm^3 of solution from the first tube, into the tube labelled x10.
f Mix thoroughly.
g Remove 1 cm^3 of well-mixed solution from the x10 tube into the x100 tube.
h Mix thoroughly.
i Repeat for x1000 and x10000 dilutions.

h Prepare 4 paper discs per pour plate as described in note 3.

i When the agar has set, turn the dish upside down. Divide the base into four sections by drawing a cross with the marker pen. Label the sections A, B, C, D.

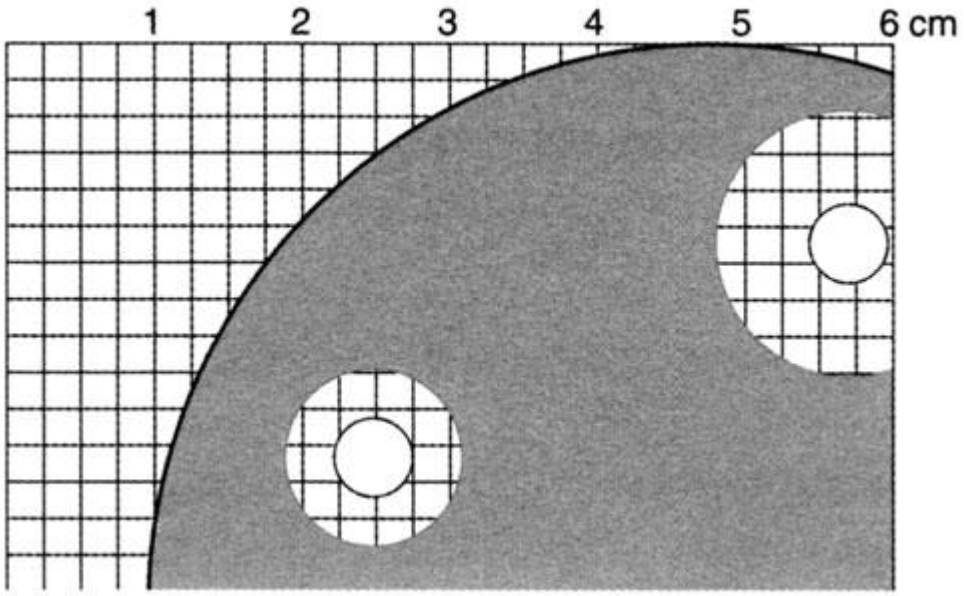
j Using sterile forceps, dip a paper disc into the plant extracts. Start with distilled water then move through the plant extracts, working from most dilute to most concentrated.

k Using sterile forceps, place a dried paper disc in each section; record the treatment of each disc. Flame forceps (or place in disinfectant) if they contact the surface of the agar and have been contaminated.

l Label the agar plate with your name and date. Tape the lid but do not seal. Incubate inverted for 2-3 days at 20-25 °C.

m Observe the plates without opening them.

n Make any measurements that will help you to compare the anti-microbial properties of the different substances. A piece of squared paper under the agar plate might be helpful here.



Teaching notes

Antiseptics are used to disinfect living tissue – both prophylactically to prevent infection and therapeutically to treat infection. Any given antiseptic is usually more effective against some microbes than others. Its activity may be affected by factors such as dilution, temperature, pH, and the presence of detergent or organic matter.

Many types of toothpaste contain low concentrations of anti-microbials, and mouthwashes claim plaque-killing potential.

Billing and Sherman's 1998 work (see web links below) explored the patterns of use of spicy cooking ingredients in hot countries and cooler countries. They examined the pattern of use of 43 ingredients and tested their antibacterial properties. The ten spices with the most potent antibacterial effects were garlic, onion, allspice, oregano, thyme, cinnamon, tarragon, cumin, cloves and lemon grass. Many spices with relatively weak antibacterial effects become much more potent when combined; examples are in chilli powder (typically a mixture of red pepper, onion, paprika, garlic, cumin and oregano) and five-spice powder (pepper, cinnamon, anise, fennel and cloves). Lemon and lime juice, while weak inhibitors themselves, also have synergistic effects.

Essential oils are also reputed to have antibacterial or antifungal properties particularly tea-tree, oil of cloves, mint and lavender. If your students investigate the antimicrobial properties of essential oils, ensure that none are ingested – many are toxic in concentrated form.

Human secretions such as sweat and tears also contain lysozyme which has antibacterial properties. Refer to CLEAPSS guidelines (Laboratory handbook section 14.4) for working with body fluids if your students investigate the properties of sweat or tears.

Students could consider the consequences for human health of this array of anti-microbial substances now available and widely used.

Health & Safety checked, May 2009

Web links

www.microbiologyonline.org.uk/teachers/resources
Society for General Microbiology – source of Basic Practical Microbiology, an excellent manual of laboratory techniques and Practical Microbiology for Secondary Schools, a selection of tried and tested practicals using microorganisms. These include *Effects of antiseptics on microbes*.

www.microbiologyonline.org.uk
MiSAC (Microbiology in Schools Advisory Committee) is supported by the Society for General Microbiology (see above) and their websites include more safety information and a link to ask for advice by email.

(Websites accessed, October 2011)

St Mary's University Twickenham London

Working scientifically skill setting up simple enquiries

GCSE AQA 6 mark question: Growing Bacteria

In this question you will be assessed on using good English, organising information clearly and using specialist terms where appropriate.
The student is told to grow some bacteria on agar jelly in a Petri dish.
Describe how the student should prepare an uncontaminated culture of bacterium in the Petri dish
You should explain the reasons for each of the steps you describe

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Marked by_____

Feedback_____

Score_____

Poor Understanding (0 marks)	Level 1 Basic Understanding (1-2 marks) **Grade 3-4**	Level 2 Clear Understanding (3-4 marks) **Grade 5-6**	Level 3 Detailed Understanding (5-6 marks) **Grade 7-8**
No relevant content	There is a brief description of how to grow bacteria	There is an attempt to explain speciation which shows an understanding of how to grow viable bacteria	There is a clear and detailed explanation of how bacteria can be grown on an agar plate
	• Knowledge of basic information • Simple understanding • The answer is poorly organised, with almost no specialist terms and their use demonstrating a general lack of understanding of their meaning, little or no detail • The spelling, punctuation and grammar are very weak	• Knowledge of accurate information • Clear understanding • The answer has some structure and organisation, use of terms has been attempted but not always accurately, some detail given • There is reasonable accuracy in spelling, punctuation, although there may still be some errors.	• Knowledge of accurate information appropriately contextualised • Detailed understanding supported by relevant evidence and examples • Answer is coherent and is in an organised, logical sequence, containing a wide range of appropriate or relevant specialist terms used accurately • The answer shows almost faultless

			spelling, punctuation and grammar

Examples of biology points made in the response

- Set up a Bunsen burner to kill any bacteria in the air around where you are working
- Only work with the petri dish under the Bunsen burner flame
- Using a sterile pipette take out some (amount in ml) of bacteria from a sample
- Squirt the sample onto the agar
- Dip the spreader into some ethanol (alcohol) and burn it off to kill any bacteria on it
- Spread the bacteria around the plate evenly
- Seal the plate in 4 places using tape
- Put a label on it indicating what it is and when it was spread
- Incubate the plate for 3 days
- Serialise used equipment

Total 6 marks

Growing Bacteria

Marks awarded for this answer will be determined by the quality of the written communication as well as the standard of the scientific response

(6 marks)

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Practical 2Investigate the effect of light intensity on the rate of photosynthesis of pondweed.

This experiment is often fiddly and challenging to get correct. Identify the control variables in the experiment and how you would adapt the method to reduce sources of error. Consider factors such as light intensity and the heat coming off the lamp.

In this practical you will:

measure the volume of oxygen produced by the pondweed as the light intensity changes as the light source is moved.

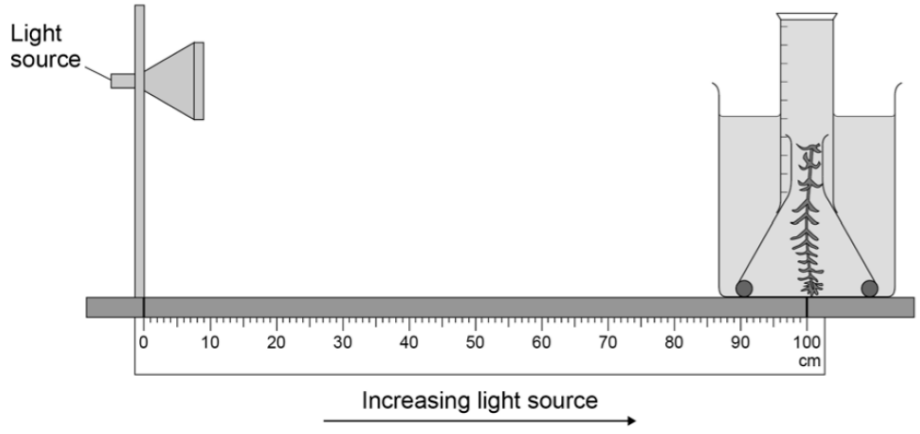
Apparatus

- a beaker
- a filter funnel
- plasticine
- a measuring cylinder (1 or 10cm^3)
- a 10 cm piece of pondweed
- a light source
- a metre rule
- a stop watch.

Method

1. Put your 10cm piece of pond weed (cut edge at top) into a beaker of water.
2. Cover the pondweed with an inverted filter funnel – raised off the bottom of the beaker with plasticine.
3. Fill the measuring cylinder with water and gently position as in the diagram.
4. Use the ruler to position the beaker of pondweed 1 metre away from the light source.

   Your experiment should look like this:



5. Start the stop watch and:
   a. count and record the number of bubbles released in three minutes
   b. record the volume of gas produced and collected in the measuring cylinder in the same three minutes.
6. Record your results in a table like this one:

	Increasing light intensity				
	100cm	80cm	60cm	40cm	20cm
Number of gas bubbles					
Volume of gas cm^3					

7. Move the light source so that the pondweed beaker is 80 cm away.
8. Refill the measuring cylinder with water and gently position as in the diagram.
   Then repeat steps 5 and 6.
9. Repeat for distances of 60, 40 and 20 cm.

Evaluation

Which method of recording gas collection was most accurate and why?

Having completed the experiment an alternative is to watch the video from David Attenborough's Green planet. Available at

https://www.bbc.co.uk/programmes/p0bg6wmg

Which would you complete and why?

How would you support students answer the questions below

Photosynthesis Required Practical

In this question you will be assessed on using good English, organising information clearly and using specialist terms where appropriate.

A student is asked to investigate the effect of light intensity on photosynthesis in pondweed. Some of the equipment they were provided with is shown below:

- a boiling tube
- freshly cut 10 cm piece of pondweed

- a light source
- a ruler
- a test tube rack
- a stop watch
- 0.2% solution of sodium hydrogen carbonate solution
- a glass rod.

Describe how they could carry out this investigation. You may wish to draw a diagram to show how the equipment can be set up.

Marked by_____

Feedback_____

Score_____

Poor Understanding (0 marks)	Level 1 Basic Understanding (1-2 marks) **Grade 3-4**	Level 2 Clear Understanding (3-4 marks) **Grade 5-6**	Level 3 Detailed Understanding (5-6 marks) **Grade 7-8**
	• Knowledge of basic information • Simple understanding • The answer is poorly organised, with almost no specialist terms and their use demonstrating a general lack of understanding of their meaning, little or no detail • The spelling, punctuation and grammar are very weak	• Knowledge of accurate information • Clear understanding • The answer has some structure and organisation, use of terms has been attempted but not always accurately, some detail given • There is reasonable accuracy in spelling, punctuation, although there may still be some errors.	• Knowledge of accurate information appropriately contextualised • Detailed understanding supported by relevant evidence and examples • Answer is coherent and is in an organised, logical sequence, containing a wide range of appropriate or relevant specialist terms used accurately • The answer shows almost faultless spelling, punctuation and grammar

Examples of biology points made in the response

When marking this question, the amounts do not need to be the same, however, the method should follow roughly the same steps

1. Set up a test tube rack containing a boiling tube at 10 cm away from the light source
2. Fill the boiling tube with the sodium hydrogen carbonate solution.
3. Put the piece of pondweed into the boiling tube with the cut end at the top. Gently push the pondweed down with the glass rod.
4. Leave the boiling tube for 5 minutes.
5. Start the stop watch and count the number of bubbles produced in one minute.
6. Repeat the count twice more. Then use the data to calculate the mean number of bubbles per minute.
7. Repeat steps **1–7** with the test tube rack and boiling tube at distances of 20 cm, 30 cm and 40 cm from the light source.

Total 6 marks

Working scientifically skill recording/analysing data
Edexcel higher paper 2 June 2018

8 (a) Figure 16 shows the effect of light intensity and temperature on the rate of photosynthesis.

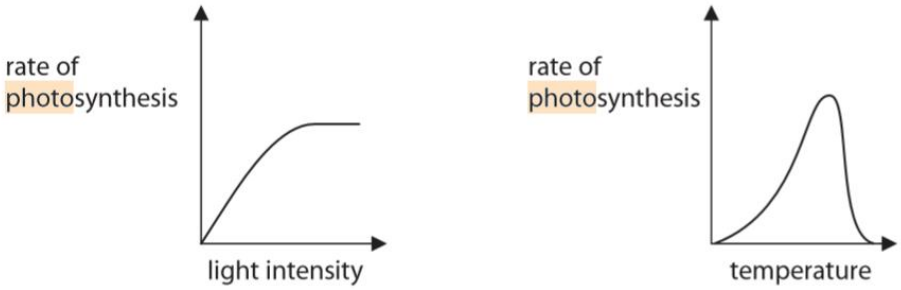


Figure 16

(i) Describe the effect of light intensity on the rate of photosynthesis.

(2)

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.....

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.....

(ii) Explain the effect of temperature on the rate of photosynthesis.

(2)

.....

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.....

(b) A student measured the rate of photosynthesis using algal balls in a laboratory. The tube of algal balls was kept at a temperature of 25 °C and was moved to different distances from a light source.

The results of this investigation showed that the rate of photosynthesis is

(1)

☒ **A** directly proportional to the distance from a light source

☒ **B** inversely proportional to light intensity

☒ **C** directly proportional to temperature

☒ **D** inversely proportional to the distance from a light source

Explain why the other options are incorrect.

Mark scheme

Question number	Answer	Additional guidance	Mark
8(a)(i)	An answer linking the following: • as light intensity increases so does the rate of photosynthesis (1) • (it levels off) when light intensity ceases to be a limiting factor (1)	 accept idea of another factor limiting the rate of photosynthesis accept named factor	**(2)** AO 3 1a AO 3 1b

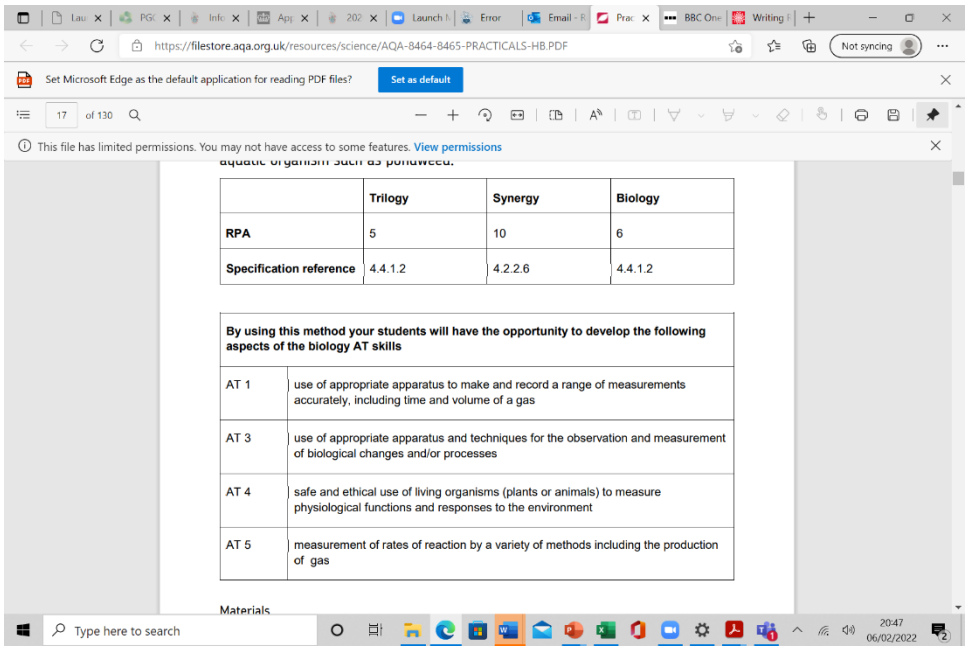
Question number	Answer		Mark
8(a)(ii)	An explanation linking **two** of the following: • as temperature increases so does the rate of photosynthesis {as enzymes can catalyse more reactions / more collisions occur} (1) • maximum rate of photosynthesis at the optimum temperature for **enzymes** (1) • {above the optimum/at high temperatures} **enzymes** become denatured (and photosynthesis decreases) (1)	 accept more enzyme-substrate complexes form accept active site changes shape for denatured	**(2)** AO 3 2a AO 3 2b

In part B the answer is D.

A is incorrect as that would suggest as the lamp is moved further from the plant the rate of photosynthesis increases.

B and C are incorrect as in the experiment changing one of these independent variables will leave the other unchanged and will eventually become a limiting factor.

Task 2
In the AQA sylabus the required skills in this practical are



	Trilogy	Synergy	Biology
RPA	5	10	6
Specification reference	4.4.1.2	4.2.2.6	4.4.1.2

By using this method your students will have the opportunity to develop the following aspects of the biology AT skills	
AT 1	use of appropriate apparatus to make and record a range of measurements accurately, including time and volume of a gas
AT 3	use of appropriate apparatus and techniques for the observation and measurement of biological changes and/or processes
AT 4	safe and ethical use of living organisms (plants or animals) to measure physiological functions and responses to the environment
AT 5	measurement of rates of reaction by a variety of methods including the production of gas

A second activity that covers these skills is reaction time. Plan an experiment that can be conducted in school that measures the effect of a factor on reaction time.,